APPLIED MICROBIOLOGY, Jan. 1968, p. 151-153

Vol. 16, No. 1
Printed in U.S.A.

NOTES

Improved Capillary-Action Replicating Apparatus

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Received for publication 18 August 1967

An ingenious device for inoculating simultaneously large numbers of cell suspensions onto a series of agar plates was described in 1965 (R. L. Massey and R. H. T. Mattoni, Appl. Microbiol. **13**:798, 1965). This device consisted of a rack holding 25 Pasteur pipettes. The pipettes were charged by capillary action when a group of tubes containing cell suspensions was raised so the tips of the pipettes contacted the liquid in the tubes. Predried plates were inoculated by raising each plate, in succession, until it contacted the pipette tips; a microdrop was discharged from each pipette onto the agar surface when the plate was lowered. The pipettes were loosely held in the rack, so the pipettes did not have to be identical in length.

The Massey and Mattoni device was inexpensive and was simple to operate, but it had two major shortcomings. First, the pipettes frequently became clogged during replication, even when 3% agar was used and the plates were carefully predried. Second, the diameter of the pipettes precluded spacing of inocula on less than 13-mm centers. This restricted the number of cell suspensions that could be examined per plate. We have eliminated these shortcomings by utilizing capillary tubes instead of Pasteur pipettes in a device patterned after the Massey-Mattoni apparatus. In the modified procedure, the inoculum is grown in medicine-dropping bottles. The droppers are used to place the inocula in the depressions of a sterile plastic tray (Fig. 1). The tray is brought up to the tips of sterile capillary tubes in a rack, to charge the tubes (Fig. 2). Plates are inoculated sequentially by carefully bringing the agar surface in contact with all capillaries, then lowering the plate.

All materials necessary for construction and operation of the replicating apparatus are readily available. Standard, 0.5-oz (14-g) medicine-dropping bottles withstand repeated sterilization, as long as the screw cap is loosened to prevent rupture of the rubber-dropper bulb. Some brands of dropping bottles (such as 2248-B, Arthur H. Thomas Co., Philadelphia, Pa.) fit into standard wire racks constructed for 20-mm diameter test tubes. The medicine dropper was convenient to emulsify a bacterial colony or a bacteriophage plaque in 1 or 2 drops of diluent for transfer to the replicating apparatus. If necessary, the colony or plaque can be isolated from its neighbors during emulsification by pressing a small cylinder (such as the Penicylinder; outer diameter, 8 mm; length, 10 mm; Fisher Scientific Co., Pittsburgh, Pa.) into the agar to form a well in which to add the emulsificant (L. O. Zwillenberg and W. Knapp, Experientia **22**:483, 1966).

Difco capillary tubes (0658-33) were the most satisfactory of the capillary tubes examined, although diSPo 20-μliter pipettes (Scientific Products Co., Evanston, Ill.) were adequate. The Difco tubes were 90 mm long and could be used as is; the diSPo pipettes, which were 125 mm long, were cut in half for use. Melting-point tubes were too large in diameter for appropriate capillary action during filling and inoculation, and were more difficult to modify for use. The size of the microdrop (inoculum) can be controlled to some degree by the diameter of the capillary tube used and possibly by adding a thixitropic solution to the inoculum. A "hook" is made by melting each capillary tube near one end (Fig. 3), so the tubes do not slip through the rack. This can be done rapidly by use of a fine-point burner, with care to make sure that the tube is not closed when it bends under its own weight during heating. We always had on hand sterile replacement tubes, in case one failed to fill during charging. Figure 3 shows a charged tube that will inoculate 15 to 20 plates before recharging is necessary.

Our apparatus (Fig. 2) was constructed largely of aluminum, so it could be loaded with capillary tubes, covered with foil, and sterilized ready for use. It consisted of two metal plates, 1 mm thick and 9 $\times$ 12 cm in area. Six plates can be clamped together in a vise and drilled simultaneously, with a wood block as the backing. As shown in Fig. 2, the plates are held by bolts onto a piece of $\frac{1}{4}$-inch (0.6-cm) aluminum, 3 inches (7.6 cm) wide and 20 inches (50 cm) long, recovered from a discarded electronic chassis facing. The aluminum is bent in the form of a "U" so the bottom of the rack is about 6 inches (15 cm) from the bench top. The holes in the plates are 2 mm or less in

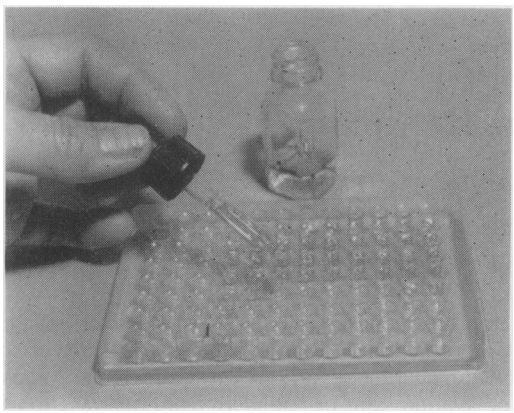

FIG. 1. *Loading the replicator tray.*

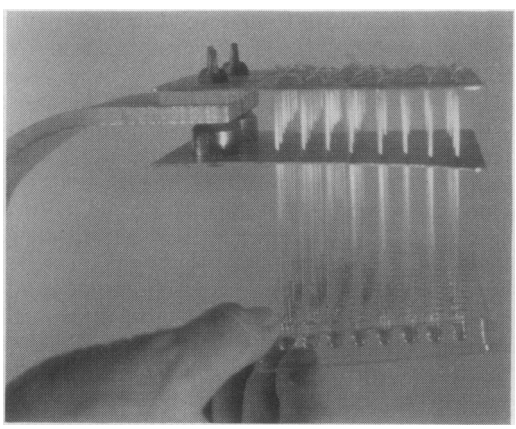

FIG. 2. *Charging the capillary tubes with inoculum.*

diameter, depending on the diameter of the capillary tubes used. A 2-mm hole accommodated all diameters of tubes examined. The holes at the tops of the plates were bevelled with a countersink; thus, the capillary tubes slid into place easily during loading. The plates are held about 1.5 inches (3.8 cm) apart for the Difco tubes or 1 inch apart for the broken diSPo pipettes. Holes can be drilled to accommodate any desired pattern.

Sterile plastic "V" plates (Cooke Engineering Co., Alexandria, Va.) were convenient (Fig. 1). Sterility is not essential if large inocula and short incubation periods are used, and a wide assortment of other plastic plates (Linbro Chemical Co., Inc., New Haven, Conn.) is available. Alternatively, the plates could be sterilized with alcohol followed by ultraviolet light (J. L. Melnick and E. M. Opton, Bull. World Health Organ. **14**:129, 1956; W. A. Rightsel, P. Schultz, D. Muething, and I. W. McLean, Jr., J. Immunol. **76**:464, 1956). An autoclavable plate of Teflon (E. Steers, E. L. Foltz, B. S. Graves, and J. Riden, Antibiot. Chemotherapy **9**:307, 1959) can be constructed, or Teflon plates containing 24 wells for round petri plates or 36 wells for square petri plates are available (Pentex, Inc., Kankakee, Ill.). The "V" plates that we used contained a pattern of 8 × 12 wells. Thus, if a pattern of 9 × 9 inocula (to place 81 inocula on a square petri plate, Fig. 4) was desired, a row from a second "V" plate had to be used. The plastic plates were sufficiently rigid so a plate support, recommended for serological work, was not necessary. It is advisable to work the capillaries up and down several times by use of the lid of a sterile petri plate before charging the capillaries; this eliminates any jamming that sometimes occurred after sterilization of the apparatus.

For bacteriophage typing of cultures, the inoculum can be placed on the agar plate first and allowed to dry for 0.5 to 1 hr; then the plate is gently flooded with a 2-ml suspension of the bacterial strain. The excess bacterial inoculum is removed, and the petri plate is allowed to dry for

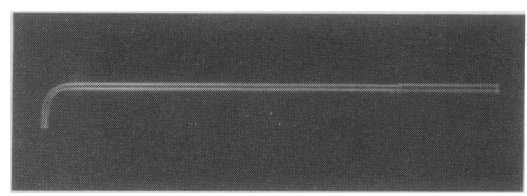

FIG. 3. *Close-up of a charged capillary tube.*

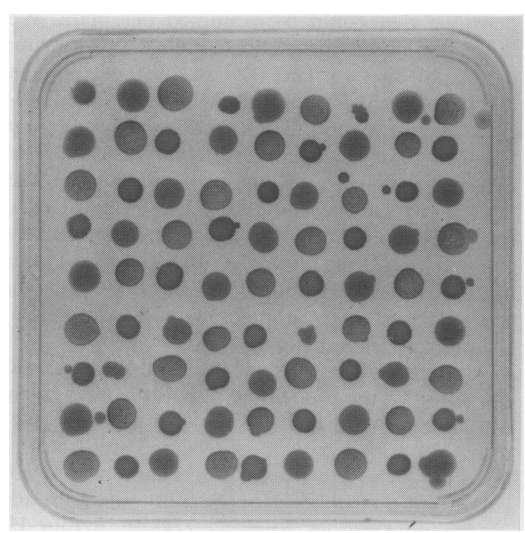

FIG. 4. *Representative plate of Serratia spp. after incubation for 3 days at 30 C.*

30 min more before incubation (H. J. Simon and S. Undseth, J. Bacteriol. **85**:1447, 1963). Other procedures that might facilitate specific applications are discussed elsewhere (P. A. Hartman, *Miniaturized Microbiological Methods*, Academic Press, Inc., New York, 1968).

Using the replicator described, we have inoculated up to 60 plates sequentially with 81 inocula per plate, the entire process taking less than 2 hr. The capillaries (Difco) were recharged every 15 plates, and the contents of the tray wells (3 drops per well) was sufficient for the entire 60 plates. Figure 4 shows a "typical" plate made during this run, with the use of *Serratia* spp. This is an "unselected" plate on 1% agar; some spattering is evident, but this does not interfere with most intended uses. For *Bacillus* spp. and other spreaders, capillaries are placed in alternate holes of the rack so that only 25 cultures can be inoculated per plate.

This investigation was supported by Public Health Service grants GM-14574 and AI-06647, and by grant GB-5568 from the National Science Foundation.